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Original article

Somatostatin Receptor tissue distribution in lung neuroendocrine tumours: a clinicopathologic and immunohistochemical study of 218 "clinically aggressive" cases.

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SUMMARY

Background. The management of pulmonary neuroendocrine tumours, with special reference to clinically aggressive carcinoids and large cell neuroendocrine carcinomas, is poorly standardized and data about somatostatin receptor expression or therapeutic guidelines for somatostatin analog administration are still debated.

Patient and methods. A series of 218 lung neuroendocrine tumours (24 metastatic typical carcinoids, 73 atypical carcinoids, 60 large cell neuroendocrine carcinomas and 61 surgically resected small cell carcinomas) was investigated for somatostatin receptor types 2A and 3 tissue distribution using immunohistochemistry, in correlation with clinicopathologic parameters, outcome, scintigraphy and treatment.

Results. Somatostatin receptors were heterogeneously distributed with a significant progressive decrease from low to high grade forms. Somatostatin receptor type 2A was strikingly over-expressed in metastatic typical carcinoids as compared to atypical carcinoids and clinically benign typical carcinoids. Somatostatin receptor tissue immunolocalization correlated with octreotide scintigraphy in 20/28 cases.

Conclusions. The immunohistochemical determination of somatostatin receptors, with special reference to low/intermediate grade tumors, may assist the clinical approach with somatostatin analog-based diagnostic and therapeutic procedures in clinically aggressive pulmonary neuroendocrine tumors.

KEY WORDS: carcinoid, immunohistochemistry, lung, neuroendocrine tumours, scintigraphy, somatostatin receptor.

Running Title: Somatostatin receptors in lung neuroendocrine tumours

Conflict of interest statement: all Authors declare the absence of any conflict of interest.

INTRODUCTION

Neuroendocrine tumours (NETs) are neoplasms mainly originating from the gastro-entero-pancreatic area (67.5%) and the bronchial tree (25.3%) [1]. According to the World Health Organization (WHO) criteria [2], lung tumours with neuroendocrine morphology include four major categories of morphologically identifiable NETs from low (typical carcinoid, TC) and intermediate grade (atypical carcinoid, AC), to high grade tumours (large cell neuroendocrine carcinoma, LCNEC, and small cell carcinoma, SCLC) that exhibit considerably different histological and clinical characteristics [3]. TCs are generally benign tumours with an excellent prognosis after radical surgery (up to 98% of 5-year survival). Regional lymph node or distant metastases are detected or develop over time in about 10% of cases. ACs are histologically similar to TCs (though with a higher mitotic count and/or necrosis), but up to 48% of cases present lymph node or distant metastases at diagnosis; their prognosis has been related to several predictive factors [4]. LCNEC and SCLC, both highly aggressive tumours, have overlapping histopathological features (except for the cell size) and clinical behaviour [5, 6].

Several neuroendocrine markers and hormonal receptors have been described in NETs having diagnostic, prognostic and also therapeutic implications. Among the latter, somatostatin receptors (SSTRs) are a family of five widely distributed G-protein-coupled receptors, that mediate different intracellular signalling pathways involved in cell proliferation, differentiation and angiogenesis [7]. Synthetic somatostatin analogs have been produced, being octreotide and lanreotide those most widely employed in the clinical practice. Their high receptor binding affinity (especially for SSTR2, SSTR3 and SSTR5) [8] is the basis for both diagnostic (i.e. scintigraphy or PET scans) and therapeutic procedure. Novel ligands with enhanced and/or wide receptor affinity (for example multivalent ligands, such as pasireotide) are currently under validation [9].

Differently from gastro-entero-pancreatic NETs, the management of pulmonary NET affected patients is less standardized apart from surgical resection [10], chemo-radiotherapy or liver

metastasis embolization [11]. In particular, diagnostic and therapeutic guidelines for somatostatin analog use in lung NETs are not available and their association with standard chemotherapy is still debated [12]. This may in part be due to the incomplete knowledge on SSTR(s) expression and distribution in pulmonary NETs: data so far generated have generally been based on polymerase chain reaction, *in situ* hybridization, autoradiography or immunohistochemistry analyses of limited and/or heterogeneous neuroendocrine tumour series, accounting for less than 100 reported cases (most of them being carcinoids) [13-20]. Recently, we reported a strong correlation between specific cellular patterns of SSTR2 immunostaining and *in vivo* scintigraphic data in a large series of gastro-entero-pancreatic and few lung neuroendocrine tumours [21], indicating that immunohistochemistry may correctly define the SSTR protein distribution in these neoplasms.

Since in the common clinical practice the presence of one or more SSTR subtypes in a given tumour (either by means of positive somatostatin analog-based nuclear imaging or SSTR immunohistochemistry) should be demonstrated to justify patient's selection for somatostatin analog therapy, this study was aimed at correlating the SSTR expression profile of a large series of "clinically aggressive" pulmonary neuroendocrine tumours (thus excluding conventional non-metastatizing typical carcinoids) with complete clinical and pathological data in order to substantiate the rationale for somatostatin analog clinical use in these tumour categories.

MATERIALS AND METHODS

Case series

In years 1989-2007, a total of 883 cases of surgically resected NETs of the lung were on record in the pathology files of the Universities of Turin and Parma and at the IEO of Milan (467, 188 and 228 cases, respectively). Among them, a series of 218 "clinically aggressive" lesions was collected (129 cases from Turin, 40 from Parma and 49 from Milan) that included 24 TC with metastases at the time of diagnosis (TC mets) [all but one in regional lymph nodes, the remaining

case to the liver], 73 AC, 60 LCNEC and 61 surgically resected SCLC. For all cases, from one to six representative haematoxylin and eosin sections were available for review and re-classification according to the WHO scheme [2]. A control group of 41 TC free from node and distant metastases at the time of diagnosis and during available follow up (control TC) were collected from the files of the University of Turin, in the same period.

Pathological material corresponded to primary lung samples in all but 14 cases, where lymph node metastases only were analyzed. For all cases, clinicopathologic information including sex, age, tumour location, surgical procedure, parenchymal location, primary tumour size, Ki-67 labeling index (expressed as the percentage of positive cells in highest labeling areas), nodal status, stage, follow up and site of distant metastases were collected. All cases were anonymized by a pathology staff member not involved in the study. Clinical data were compared and analysed through coded data, only. The study was approved by the institutional review board of the Hospital.

In 28 cases, [^{111}In-DTPA^{0}]-octreotide scintigraphic data (Octreoscan) were available for correlation analyses (eight of these cases had been included in a previous report [19]). Somatostatin receptor scintigraphy findings were scored into four groups, according to Kwekkeboom *et al* [22] criteria.

Finally, eight cases were submitted to somatostatin analog based therapy (intramuscular injection of octreotide for six months, 20 mg/28 days, and/or 90Y-DOTATOC radiometabolic therapy, either alone or in combination with chemotherapeutic agents) and information on response to treatment (in terms of partial remission, stable disease or progressive disease) were available.

SSTR immunohistochemistry

Immunohistochemistry was performed using polyclonal antibodies against SSTR type 2A, (diluted 1/3000, BioTrend Cologne, Germany), type 3 (diluted 1/1000, AbCam, Cambridge, UK) and type 5 (diluted 1/500, AbCam) according to previously described protocols [21]. The specificity

of the reactions was validated in parallel control sections stained by omitting the primary antibodies for each immunohistochemical run. Pancreatic islets served as positive controls.

The efficiency of all three antibodies was tested in a pilot series of 56 randomly selected cases. SSTR5 immunohistochemistry in positive controls and test sections proved unsatisfactory in terms of both intensity and background, and was therefore not investigated further in the whole series.

SSTR2A and SSTR3 staining evaluation

Immunohistochemical stains for SSTR type 2A and type 3 were scored as recently proposed by our group [21]. This semi-quantitative scoring system applies better for SSTR2A and takes into consideration both the subcellular localization and the extent of the staining, as follows: score 0: absence of immunoreactivity; score 1: pure cytoplasmic immunoreactivity, either focal or diffuse; score 2: membranous reactivity in less than 50% of tumour cells, irrespective of the presence of cytoplasmic staining; score 3: circumferential membranous reactivity in more than 50% of tumour cells, irrespective of the presence of cytoplasmic staining.

SSTR3 immunohistochemical reaction showed a diffuse cytoplasmic staining, with weak membrane reinforcement in some cases, either of control or of test series. Reactions were evaluated in a four-tier scoring system, from 0 (no reaction) to 3, according to the intensity of cytoplasmic staining and irrespective of the extent of positive neoplastic cells.

Statistical analysis

For statistical purposes, immunohistochemical scores 2 and 3 of both SSTR type 2A and type 3 antibody were grouped together and considered positive, while scores 0 and 1 were considered negative. Chi-square and One-way ANOVA tests were used to compare clinicopathologic data and SSTR(s) distribution among different tumour categories, setting the level of statistical significance of $p=0.05$. Concondance analysis between immunohistochemical results

and scintigraphy data was investigated using a contingency table analysis. Univariate survival analysis among different tumour categories and with respect to SSTR(s) expression was based on the Kaplan-Meier product limit estimate of overall survival distribution. Unadjusted differences between survival curves were tested using the LogRank test.

RESULTS

Clinicopathologic findings

Clinicopathologic features of the 218 cases of "clinically aggressive" lung NETs are summarized in Table 1.

Male predominance was observed in high grade carcinomas as opposed to low and intermediate grade tumours ($p<0.0001$), whereas no significant difference among the different tumour types was observed in terms of patient age, tumour location and surgical procedure applied.

With respect to pulmonary location, a significant difference was observed among the four groups ($p=0.02$), being central location predominant in TC mets, as compared to AC and LCNEC which presented a more frequent peripheral onset, whereas SCLC were equally distributed among the two locations.

The mean primary tumour size significantly increased from TC mets to AC and poorly differentiated tumours ($p=0.003$), as well as the mean Ki-67 proliferative index ($p<0.0001$). The rate of nodal involvement at the time of diagnosis was 47%, 39% and 61% in AC, LCNEC and SCLC respectively, with no difference between N1 *vs* N2-3 location of positive lymph nodes.

Overall survival was strongly depending on tumour type (Figure 1, $p<0.0001$) with no disease-related deaths for the metastatic TC group, and a mean overall survival of 122, 30 and 23 months for AC, LCNEC and SCLC, respectively.

With respect to the presence of ectopic peptide secretion, all cases were clinically non-functioning, except for two cases of AC with ectopic ACTH secretion in the presence of diabetes mellitus and metabolic disorders.

Immunohistochemical distribution of SSTR2A and SSTR3

All immunohistochemical reactions were analyzed independently by two observers (LR and MV) in one centre (Turin) according to the previously described scoring system. A satisfactory reproducibility was obtained in the vast majority of cases and the few discrepant cases were re-scored at a multi-head microscope and a consensus was reached. SSTR2A staining pattern showed a strong membrane positivity in TC mets and AC groups as opposed to high grade carcinoma groups, in which membrane staining was found in focal areas or scattered cells only (Figure 2A). SSTR3 showed cytoplasmic reactivity with different staining intensity and only rare cases showed a membrane pattern of staining. SSTR types 2A and 3 distribution among the different tumour types is illustrated in Figure 2B. SSTR type 2A was expressed in 34% of control TC, 71% of metastatic TC, 51% of AC, 33% of LCNEC and 38% of SCLC. Similarly, SSTR type 3 showed a progressive decrease of positive reaction from low to intermediate to high grade tumour cases, being significantly expressed in 66% of control TC, 58% of metastatic TC, 45% of AC, 33% of LCNEC and 29% of SCLC. Grouping together low/intermediate grade (TC mets and AC) and high grade cases (LCNEC and SCLC) the difference of SSTR2A and SSTR3 expression was 56% *vs* 36% and 48% *vs* 31%, respectively (Figure 2B).

Detailed analysis of SSTR type 2A and type 3 immunohistochemical distribution in correlation with the different tumour types (Table 2) revealed a significantly higher expression of both SSTR2A and SSTR3, either alone or in combination, in the low to intermediate grade tumour group (TC mets + AC) as compared to high grade carcinomas (LCNEC + SCLC). Comparing the different groups individually, TC mets showed a significantly higher expression as compared to control (non metastatic) TC, in terms of both SSTR2A expression alone and in the presence of

SSTR2A and SSTR3 co-expression. The prevalence of SSTR2A expression in TC mets was also higher as compared to AC, although just below statistical significance. AC showed a significantly higher prevalence of SSTR2A expression and SSTR2A/SSTR3 co-expression as compared to LCNEC, and a lower expression of SSTR3 as compared to control TC. Interestingly, similar results for both SSTR2A and SSTR3 expression prevalence were observed in LCNEC and SCLC groups.

Correlation between SSTR2A and SSTR3 immunohistochemistry with clinicopathologic parameters.

No significant correlation was found in the group of either low and intermediate grade tumors (TC mets + AC) or high grade carcinomas (LCNEC + SCLC) tumours correlating SSTR2A and SSTR3 expression with clinical and pathological parameters, except for a higher prevalence of SSTR3 positive cases in the N+ group (without distinction of N1 or N2-3 lymph node station) of poorly differentiated carcinomas (*p=0.01*). No association with clinical behaviour and outcome was observed for SSTR(s) expression in any tumour type. Furthermore, no correlation was observed between mean Ki-67 and SSTR(s) expression evaluated within each tumour category (data not shown).

Correlation between SSTR2A and SSTR3 immunohistochemistry with *in vivo* octreotide scintigraphy data.

In 28 cases, octreotide scintigraphy data were available either performed pre-operatively or, most often, post-operatively when a metastatic event occurred. Comparing the scintigraphy data with SSTR(s) immunohistochemistry, an overall SSTR2A immunohistochemistry/octreotide scintigraphy agreement of 64% (18/28 cases) was reached. Different concordance rates were observed in the group of low and intermediate grade tumours (69%) and poorly differentiated carcinomas (40%). Correlation of SSTR3 antibody reactivity with *in vivo* data was less striking with an overall agreement of 50%. The immunohistochemical presence of at least one receptor type was

concordant with scintigraphy in 20/28 cases (71% of agreement), although with a marked loss of "specificity" (Table 3).

With respect to biotherapy response, 8 cases, all but one affected by AC, received at the time of disease progression long-acting octreotide and/or therapy with radiolabeled octreotide, both associated or not to standard platinum-based chemotherapy. SSTR2A immunoreactivity correlated with response to treatment in all but one cases (7/8); among these, three patients had disease stabilization in the presence of SSTR2A expression, whereas the remaining four cases, completely lacking SSTR2A reactivity, had disease progression. The single discrepant case had disease progression in spite of SSTR2A immunohistochemical expression. Notably, octreotide scintigraphy correlated with somatostatin analog response in 50% of cases (4/8), only.

DISCUSSION

In the present study, we analyzed the tissue distribution of SSTR2A and SSTR3 in a large series of pulmonary neuroendocrine neoplasms consisting of low to intermediate grade tumours with "clinically aggressive" features (i.e. lymph node or distant metastases) and high grade large cell or small cell carcinomas. Although the link between SSTR(s) expression and lung NETs management was already proposed [7, 23], an extensive analysis of SSTR tissue distribution is missing, being data available for limited series, with heterogeneous techniques and mostly restricted to clinically benign carcinoids (usually not requiring further treatment) [13, 14, 18, 19].

From the literature data, the current knowledge on SSTRs and lung NETs can be summarized as follows: i) lung NETs contain SSTR subtypes having an affinity to octreotide, namely types 2, 3 and 5, with a decreasing expression from low/intermediate to high grade tumours [23]; ii) somatostatin receptor scintigraphy may be used to localize neuroendocrine tumours primary or secondary to the lung [24], although its role is supported by limited literature data, most often biased by heterogeneous imaging modalities, small case series and unselected tumour types [25,

26]; iii) biotherapies with somatostatin analogs are largely employed for advanced NETs of the gastro-entero-pancreatic tract, either alone or in combination with interferon or chemotherapeutic agents [12], but in lung NETs the experience is restricted to limited clinical studies using somatostatin analogs as drugs [11, 27] or radionuclide vectors [28]. In summary, compared to gastro-entero-pancreatic NETs, the management of aggressive forms of lung NETs is not well clarified yet and the role of biotherapies is incompletely explored.

In this study, a complete map of SSTR type 2A and type 3 tissue distribution was provided in a large series of lung NETs including the whole spectrum of clinically aggressive forms (from metastatic typical carcinoids to small cell lung cancer).

Both SSTR types 2A and 3 had a decreased expression from low/intermediate to high grade tumours, as previously observed by our group in smaller series [18, 19]. Interestingly, in the group of low to intermediate grade tumours, SSTR2A was expressed at a significant higher frequency in metastatic TC as compared either to clinically benign control TCs, alone (*p=0.004*) or in co-expression with SSTR3 (*p=0.04*), either compared to the AC group. The Ki-67 index of TC with metastases was not different from that of non metastatic TC (data not shown), suggesting that the SSTR2A status rather than the proliferation fraction is related to metastatic propensity in lung carcinoids. Furthermore, a higher expression of intracellular molecules downstream to the SSTRs, such as mTOR and its effector p70S6K, was observed by our group in a series of metastatic TC as compared to non-metastatizing TC (manuscript in preparation), further supporting a biological diversity among these two subgroups. No data on the functional role of somatostatin receptors in favouring tumor metastases are present in the literature that might support this observation, that apparently contrasts with the decrease of somatostatin receptor expression in high grade – frequently metastatic - tumors and whose biological meaning would need further investigation. Moreover, it should be underlined that our series of metastatic TC had an excellent prognosis since no patient (even the single case with liver metastases) is currently dead of the disease, thus confirming that nodal involvement in typical carcinoids is not significantly impacting on survival

(aggiungere citazione Ferolla) and raising the question of the potential clinical benefit of post-surgical somatostatin analog therapy in asymptomatic patients.

When comparing the present immunohistochemical results with the prevalence data of positive octreotide scintigraphy in lung NETs available from the literature, a slightly higher percentage is reported for carcinoids (about 70% of primary tumours in one of the most recent and complete series – 28 cases) [29], while an exceedingly higher percentage is reported for SCLC, (up to 96% of primary tumours in a large, old series – 100 cases) [30]. In the present series of 28 cases with available scintigraphy data, an overall 71% agreement between Octreoscan and immunohistochemistry was obtained. Major causes of discrepancy between these two methods include on the one hand false positive SSTRs scintigraphy related to necrotic areas or inflammation (often observed in SCLC) [19], in the other the possible role of other SSTR subtypes than types 2A and 3 with high affinity for the currently used somatostatin analogues (namely SSTR5, not analyzed in this study for technical reason). Moreover, it should be speculated that immunohistochemistry may represent a less sensitive method as compared to somatostatin receptor scintigraphy because of heterogeneous SSTR distribution in tumour tissues or technical artifacts related to tissue fixation. However, we strongly believe that speculating which method is more sensitive or specific is indeed useless. In contrast, we encourage the use of immunohistochemistry as a potentially useful adjunct to SSTR scintigraphy in lung NETs work-up.

Despite several cases in our series displayed an aggressive and fatal clinical course even in the group of low/intermediate grade tumours, only eight patients of the present series underwent biotherapy with somatostatin analogs, therefore limiting the possibility to test the possible value of somatostatin receptor immunohistochemistry to predict the clinical response to somatostatin analog therapy. This fact, rather than a limit of our investigation, represents the actual clinical picture and reflects the lack of robust trials validating the use of somatostatin analogs in advanced lung NETs.

In conclusion, our study describes SSTR(s) tissue localization in a very large series of aggressive lung NETs with clinicopathologic correlates, and strengthens the concept that the

information on SSTR(s) expression at the tissue level in lung NET patients developing clinically aggressive disease may improve the clinical approach with somatostatin analog-based diagnostic and therapeutic procedures.

Acknowledgements: We are grateful to Prof. G. Bussolati (Pathology Unit, University of Turin), Prof. F. Ardissone (Thoracic Surgery, University of Turin at San Luigi Hospital) and Prof. M. Rusca (Thoracic Surgery, University of Parma) for their helpful suggestions. We are also indebted to drs A. Berruti, M.P. Brizzi, G. Selvaggi (Oncology Unit, University of Turin at San Luigi Hospital) and dr M. Tiseo (Oncology Unit, University Hospital of Parma) for providing clinical information on octreotide treatments.

Financial support: Work supported by grants from the Italian Ministry of University and Research, Rome (ex-60% to MP and MV; PRIN 2006 to MP, FIL 2007 to GR) and by an unrestricted research grant from Novartis Oncology to MP (Origgio, Italy)

REFERENCES

1. Modlin IM, Kidd M, Latich I et al. Current status of gastrointestinal carcinoids. Gastroenterology 2005; 128: 1717-1751.

2. Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC. Tumours of the Lung, Pleura, Thymus and Heart. Lyon: IARC Press 2004.

3. Gustafsson BI, Kidd M, Chan A et al. Bronchopulmonary neuroendocrine tumours. Cancer 2008; 113: 5-21.

4. Beasley MB, Thunnissen FB, Brambilla E et al. Pulmonary atypical carcinoid: predictors of survival in 106 cases. Hum Pathol 2000; 31: 1255-1265.

5. Iyoda A, Hiroshima K, Nakatani Y, Fujisawa T. Pulmonary large cell neuroendocrine carcinoma: its place in the spectrum of pulmonary carcinoma. Ann Thorac Surg 2007; 84: 702-707.

6. Takei H, Asamura H, Maeshima A et al. Large cell neuroendocrine carcinoma of the lung: a clinicopathologic study of eighty-seven cases. J Thorac Cardiovasc Surg 2002; 124: 285-292.

7. Volante M, Bozzalla-Cassione F, Papotti M. Somatostatin receptors and their interest in diagnostic pathology. Endocr Pathol 2004; 15: 275-291.

8. Patel YC, Srikant CB. Subtype selectivity of peptide analogs for all five cloned human somatostatin receptors (hsstr 1-5). Endocrinology 1994; 135: 2814-2817.

9. van der Hoek J, van der Lelij AJ, Feelders RA et al. The somatostatin analogue SOM230, compared with octreotide, induces differential effects in several metabolic pathways in acromegalic patients. Clin Endocrinol (Oxf) 2005; 63: 176-184.

10. McMullan DM, Wood DE. Pulmonary carcinoid tumours. Semin Thorac Cardiovasc Surg 2003; 15: 289-300.

11. Kosmidis PA. Treatment of carcinoid of the lung. Curr Opin Oncol 2004; 16: 146-149.

12. Oberg K. Chemotherapy and biotherapy in the treatment of neuroendocrine tumours. Ann Oncol 2001; 12 Suppl 2: S111-114.

13. Reubi JC, Waser B, Sheppard M, Macaulay V. Somatostatin receptors are present in small-cell but not in non-small-cell primary lung carcinomas: relationship to EGF-receptors. Int J Cancer 1990; 45: 269-274.

14. Reubi JC, Kappeler A, Waser B et al. Immunohistochemical localization of somatostatin receptors sst2A in human tumours. Am J Pathol 1998; 153: 233-245.

15. Janson ET, Stridsberg M, Gobl A et al. Determination of somatostatin receptor subtype 2 in carcinoid tumours by immunohistochemical investigation with somatostatin receptor subtype 2 antibodies. Cancer Res 1998; 58: 2375-2378.

16. Kimura N, Pilichowska M, Date F et al. Immunohistochemical expression of somatostatin type 2A receptor in neuroendocrine tumours. Clin Cancer Res 1999; 5: 3483-3487.

17. Reubi JC, Waser B, Liu Q et al. Subcellular distribution of somatostatin sst2A receptors in human tumours of the nervous and neuroendocrine systems: membranous versus intracellular location. J Clin Endocrinol Metab 2000; 85: 3882-3891.

18. Papotti M, Croce S, Macri L et al. Correlative immunohistochemical and reverse transcriptase polymerase chain reaction analysis of somatostatin receptor type 2 in neuroendocrine tumours of the lung. Diagn Mol Pathol 2000; 9: 47-57.

19. Papotti M, Croce S, Bello M et al. Expression of somatostatin receptor types 2, 3 and 5 in biopsies and surgical specimens of human lung tumours. Correlation with preoperative octreotide scintigraphy. Virchows Arch 2001; 439: 787-797.

20. Zatelli MC, Maffei P, Piccin D et al. Somatostatin analogs in vitro effects in a growth hormone-releasing hormone-secreting bronchial carcinoid. J Clin Endocrinol Metab 2005; 90: 2104-2109.

21. Volante M, Brizzi MP, Faggiano A et al. Somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumours: a proposal of scoring system correlated with somatostatin receptor scintigraphy. Mod Pathol 2007; 20: 1172-1182.

22. Kwekkeboom DJ, Teunissen JJ, Bakker WH et al. Radiolabeled somatostatin analog [177Lu-DOTA0,Tyr3]octreotate in patients with endocrine gastroenteropancreatic tumours. J Clin Oncol 2005; 23: 2754-2762.

23. Pelosi G, Volante M, Papotti M et al. Peptide receptors in neuroendocrine tumours of the lung as potential tools for radionuclide diagnosis and therapy. Q J Nucl Med Mol Imaging 2006; 50: 272-287.

24. Ameri P, Gatto F, Arvigo M et al. Somatostatin receptor scintigraphy in thoracic diseases. J Endocrinol Invest 2007; 30: 889-902.

25. Koopmans KP, de Vries EG, Kema IP et al. Staging of carcinoid tumours with 18F-DOPA PET: a prospective, diagnostic accuracy study. Lancet Oncol 2006; 7: 728-734.

26. Hillel PG, van Beek EJ, Taylor C et al. The clinical impact of a combined gamma camera/CT imaging system on somatostatin receptor imaging of neuroendocrine tumours. Clin Radiol 2006; 61: 579-587.

27. De Dosso S, Bajetta E, Procopio G et al. Pulmonary carcinoid tumours: indolent but not benign. Oncology 2007; 73: 162-168.

28. van Essen M, Krenning EP, Bakker WH et al. Peptide receptor radionuclide therapy with 177Lu-octreotate in patients with foregut carcinoid tumours of bronchial, gastric and thymic origin. Eur J Nucl Med Mol Imaging 2007; 34: 1219-1227.

29. Granberg D, Sundin A, Janson ET et al. Octreoscan in patients with bronchial carcinoid tumours. Clin Endocrinol (Oxf) 2003; 59: 793-799.

30. Reisinger I, Bohuslavitzki KH, Brenner W et al. Somatostatin receptor scintigraphy in small-cell lung cancer: results of a multicenter study. J Nucl Med 1998; 39: 224-227.

Table 1. Clinicopathologic features of 218 "clinically aggressive" pulmonary neuroendocrine tumours.

	TC mets (#24) *No.a.*		**AC (#73)** *No.a.*		**LCNEC (#60)** *No.a.*		**SCLC (#61)** *No.a.*	
Sex	24		73		60		61	
M		12		42		53		49
F		12		31		7		12
Age (y)	24		73		60		61	
Range		15-78		11-77		35-87		44-84
Mean		48		55		64		65
Median		49		57		65		66
Tumour location	23		66		49		45	
RUL		2		18		17		14
RML		5		18		8		3
RIL		3		10		5		11
LUL		4		10		12		9
LIL		9		10		7		8
Surgical procedure	23		70		54		59	
Pneumonectomy		3		4		4		4
Bilobectomy		2		5		1		1
Lobectomy		17		55		44		39
Wedge resection		1		5		2		5
Lymph node biopsy		0		1		3		10
Parenchymal location	15		36		32		22	
Central		9		10		8		12
Peripheral		4		24		22		10
Multifocal		2		2		2		0
Primary tumour size (mm)	23		62		51		47	
≤10		1		6		2		0
11-29		14		24		16		15
≥30		8		32		33		32
Mean		25		32		42		38
Ki67 (%)	18		49		39		44	
Mean		3		16		70		76
Range		0,3-8		1-70		30-90		40-95
Nodal status	23		61		49		57	
N0		0		32		30		22
N1		16		16		11		14
N2-3		7		13		8		21
Stage	23		57		50		51	
1A-1B		0		13-17		9-14		9-11
2A-2B		9-6		6-8		1-12		3-9
3A-3B		8-0		9-4		7-3		15-2
4		0		0		4		2
Follow-up (mos)	24		72		58		61	
Follow up time: range		2-175		1-215		1-214		1-120

mean Mean OS		47 *n.r*		57 122		41 30		27 23
Disease status NED/DOC AWD DOD	24	 22 2 0	72	 47 5 20	58	 20 0 38	61	 21 0 40
Site of distant metastases		Liv: 1 Lu: 1		Adr: 1 BM: 1 Bo: 3 CNS: 1 Liv: 5 Lu: 1 Med: 2 Ov:1 Pc:1 Thy:1		Bo: 4 ChW: 1 CNS: 1 Liv: 3 Lu: 3		Adr: 1 AxLN:1 Bo: 6 ChW: 1 CNS: 7 Liv: 9 Lu: 6 Med: 3

Abbreviations: TC mets: typical carcinoid with metastases; AC: atypical carcinoid; LCNEC: large cell neuroendocrine carcinoma; SCLC: small cell lung carcinoma; No.a.= number of available cases; M: male; F: female; RUL: right upper lobe; RML: right medium lobe; RIL: right inferior lobe; LUL: left upper lobe; LIL: left inferior lobe; n.a= not applicable; mos= months; NED: not evidence of disease; DOC: death of other causes; AWD: alive with disease; DOD: death of disease; OS: overall survival; nr: not reached; Adr: adrenal gland; AxLN: axillary lymph node; BM: bone marrow; Bo: bone; ChW: chest wall; CNS: central nervous system; Liv: liver; Lu: lung; Med: mediastinum; Ov: ovary; Pc: pancreas; Thy: thyroid.

Table 2. Correlation between different tumour types and SSTR2A and SSTR3 immunohistochemical distribution in lung NETs.

	SSTR2		X^2 p	SSTR3		X^2 p	SSTR 2 and/or 3		X^2 p	SSTR 2 and 3		X^2 p
	pos	**neg**		**pos**	**neg**		**pos**	**neg**		**pos**	**neg**	
Control TC (41)	**14**	**27**	**0.004**	**27**	**14**	0.5	**32**	**9**	0.6	**9**	**32**	**0.04**
TC mets (24)	**17**	**7**		**14**	**10**		**20**	**4**		**11**	**13**	
Control TC (41)	**14**	**27**	0.08	**27**	**14**	**0.03**	**32**	**9**	0.06	**9**	**32**	0.2
AC (73)	**37**	**36**		**33**	**40**		**46**	**27**		**24**	**49**	
TC mets (24)	**17**	**7**	0.08	**14**	**10**	0.3	**20**	**4**	0.09	**11**	**13**	0.2
AC (73)	**37**	**36**		**33**	**40**		**46**	**27**		**24**	**49**	
AC (73)	**37**	**36**	**0.04**	**33**	**40**	0.2	**46**	**27**	0.2	**24**	**49**	**<0.001**
LCNEC (60)	**20**	**40**		**20**	**40**		**32**	**28**		**7**	**53**	
LCNEC (60)	**20**	**40**	0.6	**20**	**40**	0.7	**32**	**28**	0.8	**7**	**53**	0.9
SCLC (61)	**23**	**38**		**18**	**43**		**34**	**27**		**7**	**54**	
TC mets + AC (97)	**54**	**43**	**0.004**	**47**	**50**	**0.01**	**66**	**31**	**0.02**	**35**	**62**	**<0.001**
LCNEC + SCLC (121)	**43**	**78**		**38**	**83**		**63**	**58**		**14**	**107**	

Abbreviations: TC: typical carcinoid; TC mets: typical carcinoid with; AC: atypical carcinoid; LCNEC: large cell neuroendocrine carcinoma; SCLC: small cell lung carcinoma; ~~WD: well differentiated; PD: poorly differentiated.~~

Table 3. Concordance between immunohistochemistry and ^{111}In-pentetreotide scintigraphy, according to somatostatin receptor type considered.

	SSTR2A	**SSTR3**	**SSTR2A or 3**
Overall agreement	**64% (18/28)**	**50% (14/28)**	**71% (20/28)**
"sensitivity"*	70%	48%	80%
"specificity"*	71%	57%	42%

FIGURE LEGENDS

Figure 1. Overall survival distribution of 213 “clinically aggressive” lung neuroendocrine tumours.

Figure 2. Distribution of SSTR2A and 3 immunoreactivity in lung NETs. Typical carcinoids with metastases (TC mets) showed strong immunohistochemical expression of SSTR2A, with usually a diffuse membrane pattern (**a**) and the higher distribution (**b**). Atypical carcinoids (AC) showed a pattern of staining similar to TC mets (**a**), but with a lower frequency of positive cases (**b**). In poorly differentiated neuroendocrine carcinomas, such as the large cell type (LCNEC), SSTR2A immunohistochemical staining showed a more patchy distribution (**a**) and the lowest overall frequency of positive cases (**b**). HE: Hematoxylin & Eosin, 200x; SSTR2A: immunoperoxidase for somatostatin receptor type 2A, 200x.

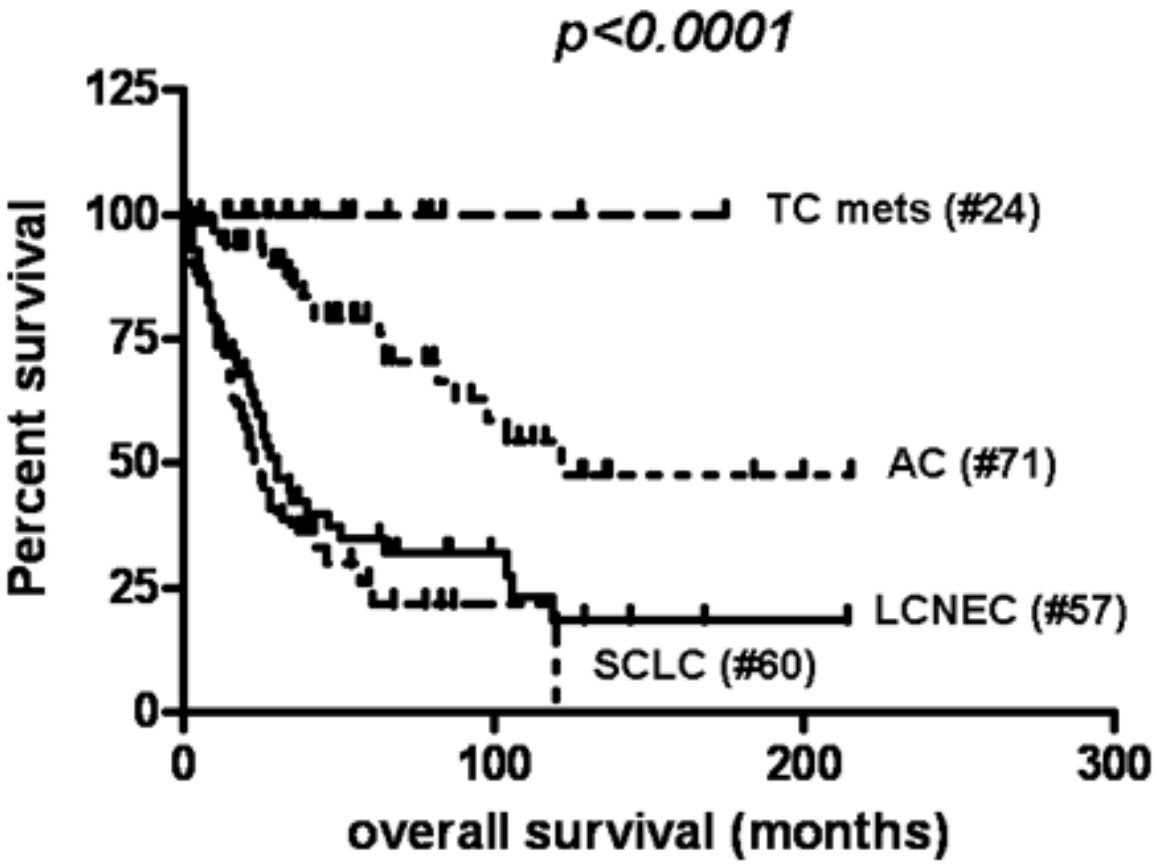
p<0.0001
125
100
75
50
25
0
Percent survival
TC mets (#24)
AC (#71)
LCNEC (#57)
SCLC (#60)
0
100
200
300
overall survival (months)

Figure 1

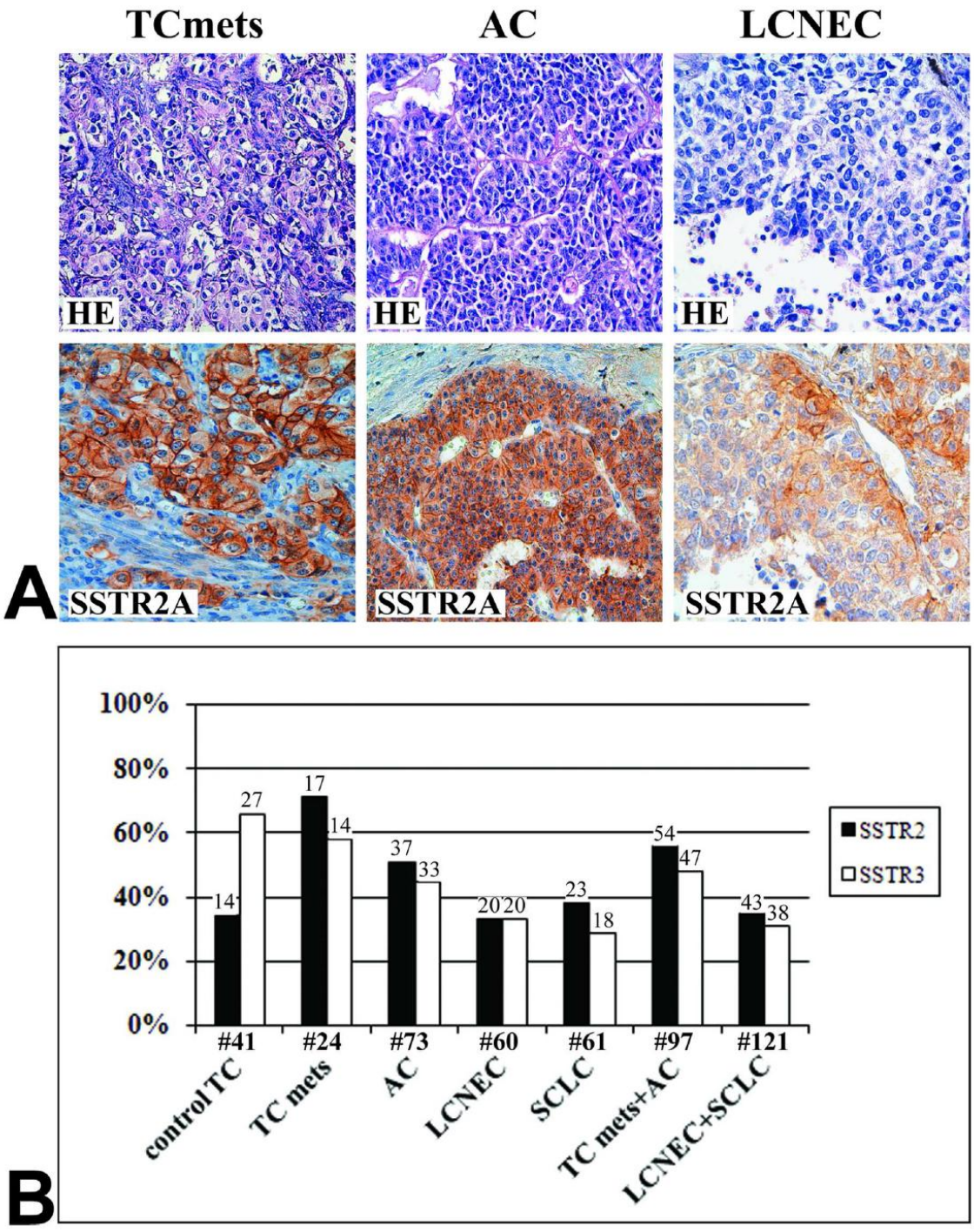
TCmets
AC
LCNEC
HE
HE
HE
SSTR2A
SSTR2A
SSTR2A
A
100%
80%
60%
40%
20%
0%
14
27
17
14
37
33
20
20
23
18
54
47
43
38
SSTR2
SSTR3
#41
#24
#73
#60
#61
#97
#121
control TC
TC mets
AC
LCNEC
SCLC
TC mets+AC
LCNEC+SCLC
B

Figure 2